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Prevalence and Antibiogram of *Staphylococcus aureus* Isolates from Non-pathological Samples of Sheep

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ABSTRACT

Staphylococcus aureus is found as commensal organism in the livestock, and they also cause opportunistic infections. They also possess various mechanisms and genes conferring them with antimicrobial resistance. The carriage of such antimicrobial resistant organisms by healthy animals poses threat to both the animal production and public health aspects. In the present study a total of 90 samples from apparently healthy sheep and their farm environment were collected. The samples consisted of skin swabs (n=30), nasal swabs (n=20), vaginal swabs (n=20) and farm environmental samples (n=20). The overall prevalence of *S. aureus* was found to be 18.8% (n=17), with the highest prevalence in skin samples. The isolates were subjected to culture sensitivity test against 17 antibiotics. The antibiogram revealed highest resistance to penicillin-G (88%), ampicillin (53%), tetracycline (47%), cefoxitin (29%) and azithromycin (29%). The isolates showed susceptibility to co-trimoxazole (94%), amikacin (82%), chloramphenicol (82%), gentamicin (76%) and gatifloxacin (76%). Out of the 17 isolates, 14 were multi-drug resistant. Such studies on prevalence and antimicrobial resistance of organisms are needed to understand the epidemiology and mechanisms of resistance. They also prove useful in formulating standard operating procedures for antimicrobial usage.

HIGHLIGHTS

- The prevalence of S. aureus was found to be 18.8% from non-pathological samples of sheep.
- The isolates showed resistance to various antibiotics and 14 out of 17 isolates were multi-drug resistant.

Keywords: *S. aureus*, Sheep, Prevalence, Antimicrobial resistance

Animal husbandry is an integral part of agricultural sector and contributes significantly to the farmers' livelihood. Sheep is an important livestock species in India because of their diverse value, which includes meat, wool, skin, and manure, all of which contribute to the agrarian economy, particularly in locations where crop and dairy production are not economically viable. Sheep husbandry serves as a source of sustenance for marginal, landless and small-scale farmers. According to the 20th livestock census, the total sheep population in the country has increased by 14.1% and currently is 74.26 million. Hence it becomes important to understand about the important pathogens affecting sheep and ways to mitigate them, to have wholesome sheep production. *Staphylococcus aureus* lives as commensal in most of the warm-blooded animals and causes opportunistic infections. According to studies, 29% of the sheep population carries *S. aureus* and can possibly act as source of transmission to humans and other species. Nasal carriage is also said to be a major reservoir in the ruminants (Peton and Le Loir, 2014). They cause mainly mastitis and septicemia in small ruminants. In lambs, they can cause secondary infections resulting in fatal toxemia and chronic abscess formation (Haag *et*

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al., 2019). They are also an important causative agent for food intoxication. *S. aureus* is also known to adapt to antibiotic selection pressure and can harbor different antimicrobial resistance genes. This in turn can enter the food chain and affect human beings. The carriage of *S. aureus* by healthy animals poses a threat of transmission to other animals and to personnel handling these animals. The transfer of antimicrobial resistant pathogen between humans and animals raises an important public health issue, which demands investigation about the prevalence of such pathogens and their antimicrobial resistance pattern. Hence the current study was undertaken to find the prevalence of *S. aureus* in healthy animals and farm premises and their antibiogram pattern.

MATERIALS AND METHODS

Sample collection

A total of 90 samples were collected from sheep farms in the state of Punjab, which consisted of 30 skin swabs, 20 nasal swabs, 20 vaginal swabs and 20 samples from farm premises. All the animals were apparently healthy and were reared in backyard of the farmers. Nasal swabs were taken from anterior part of the nasal cavity and skin swabs were preferably obtained from axillary region, inguinal region, and skin folds. Farm premises samples were collected from manger, bedding material and floor. The samples were collected in a sterile cotton swab and transported in ice to the laboratory for further processing.

Isolation of *Staphylococcus aureus*

The samples were inoculated on Brain Heart Infusion agar (HiMedia, Mumbai) for the primary isolation of bacteria. The plates were incubated for 18-24 h at 37 ºC and the growth was observed. The suspected colonies were subjected to Gram's staining and checked for the isolates showing Gram positive cocci appearance. The colonies which were presumptively identified as *Staphylococcus* species, were further streaked on Mannitol Salt Agar (HiMedia, Mumbai) and incubated for 18-24h at 37 ºC. The colonies which showed yellow colour in the agar were considered to be *S. aureus*. The colonies were also confirmed bio-chemically by testing for catalase and coagulase production.

PCR confirmation of *S. aureus*

Polymerase Chain Reaction (PCR) for the gene *nuc*, which is species specific for S. aureus, was used to confirm that the isolates were *S. aureus*. DNA from the isolates were extracted using snap chilling method. The primer sequence is mentioned in Table 1. The reaction mixture was made with 12.5 µl of GoTaq® green mastermix, 1 µl of forward primer, 1 µl of reverse primer, 5 µl of the test DNA and the final volume was made to 25 µl by adding nuclease free water. The PCR reaction was performed as per the protocol by Brakstad *et al*., (1992). The cycling conditions were set as follows: the first step of initial denaturation for 5 minutes at 94 ºC; the step consisted of denaturation, annealing and extension for 1 minute at 94 ºC, 30 seconds at 55 ºC and 1.5 minutes at 72 ºC respectively; the final extension for 3.5 minutes at 72 ºC.

Table 1: Primers for the confirmation of *S. aureus*

Gene	Primer sequence - 5' - 3'	Amplicon Size	Reference
nuc	F-GCGATTGATGGT GATACGGTT R-AGCCAAGCCTTGA CGAACTAAAGC	279 bp	(Brakstad *et al*., 1992)

Antibiogram of *S. aureus*

The isolates were examined for phenotypic antimicrobial susceptibility for 17 antibiotics by Kirby-Bauer disc diffusion method. The isolates were grown overnight in BHI broth, and they were matched with 0.5 McFarland standard. The broth containing the isolates were streaked on Mueller Hinton Agar at all directions. The antibiotic discs were placed at equal distances from each other and incubated at 37°. The zone diameters were measured and were interpreted according to Clinical and Laboratory Standards Institute (CLSI, 2017) guidelines.

RESULTS AND DISCUSSION

The present study was conducted to understand about the prevalence of *S. aureus* in the non-pathological samples of apparently healthy sheep and their environmental samples and also to study about their antimicrobial susceptibility. From the total 90 samples, *S. aureus* could be isolated from 17 samples, by morphological (Fig. 1) and cultural

(Fig. 2) methods. The isolates were confirmed to be *S. aureus* by PCR.

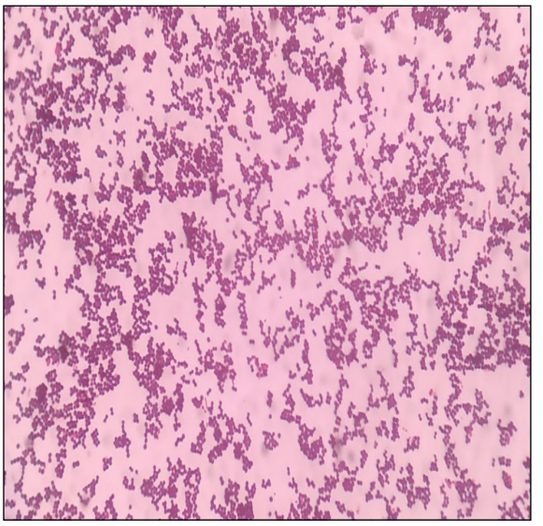

Fig. 1: *S. aureus* on Gram's staining showing characteristic appearance like bunch of grapes

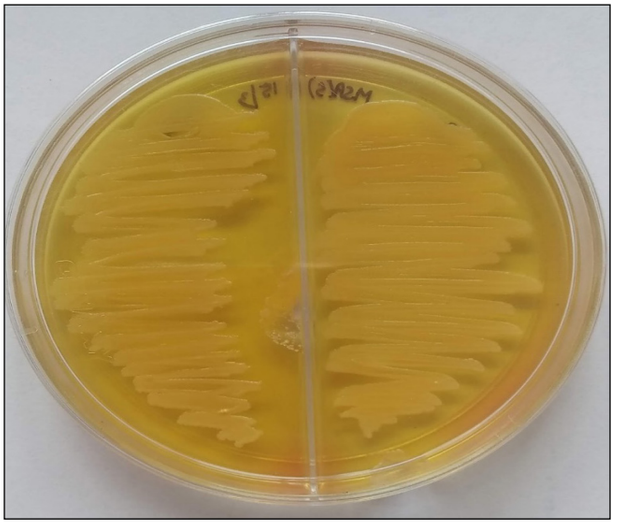

Fig. 2: *S. aureus* on Mannitol Salt Agar showing yellow-coloured colonies

The details of positive isolates distribution are mentioned in Table 2. Skin swabs showed highest prevalence of *S. aureus* isolates, followed by nasal swabs and environmental samples. Gharsa *et al*. (2012) found a prevalence of 44% of *S. aureus* in the nasal swabs of healthy sheep. In the present study 20% of the samples from farm environmental samples, like bedding and manger, showed *S. aureus* isolates. The significance of farm environmental samples harboring various pathogens were also reported in previous studies. Kalupahana *et al*. (2019) isolated methicillin resistant *S. aureus* (MRSA) from pig farm dust samples. Schnitt *et al*. (2020) also isolated *S. aureus* from pig and cattle farm environmental samples and farm equipment.

Table 2: Details of *S. aureus* positive samples

Sl. No.	Sample	Number of samples	Number of positive isolates	Percentage of positive isolates
1	Skin	30	7	23%
2	Nasal	20	4	20%
3	Vaginal	20	2	10%
4	Farm premises	20	4	20%
Total		**90**	**17**	**100%**

The antimicrobial sensitivity for the isolates was tested against 17 antibiotics and were interpreted. The overall sensitivity pattern is mentioned in Table 3. Highest resistance was shown to the following antibiotics *viz.,* penicillin-G (88%), ampicillin (53%), tetracycline (47%), cefoxitin (29%) and azithromycin (29%). The isolates showed high susceptibility to co-trimoxazole (94%), amikacin (82%), chloramphenicol (82%), gentamicin (76%) and gatifloxacin (76%). Out of 17 isolates, only 5 showed resistance to cefoxitin. The graphical representation of the antimicrobial susceptibility pattern is shown in Fig. 3, where X – axis shows different antibiotics and Y- axis shows the number of isolates.

The isolates showing resistance to three or more than three drugs are considered to be multi-drug resistant (MDR) isolates. In the present study, 14 isolates showed multi drug resistance which is 82%. This shows high rate of multi drug resistance among the bacteria. Omoshaba *et al*. (2020) found a prevalence of 10% of *S. aureus* in nasal swabs of sheep. The study also showed that the isolates were resistant to ampicillin (100%), but in contrast to our study, the isolates showed 100% resistant to co-trimoxazole. Sharma *et al*. (2011) reported 13% positivity from sheep milk samples and the isolates resistance to kanamycin, erythromycin and amoxycillin. In contrast, the isolates were susceptible to ampicillin.

Table 3: Antimicrobial sensitivity pattern of the isolates

Sl. No.	Antibiotics	Concentration of the disc used	Number of isolates (n)		
			Sensitive	Intermediate	Resistant
1	Amikacin	30 mcg	14	–	3
2	Ampicillin	10 mcg	8	–	9
3	Azithromycin	15 mcg	8	4	5
4	Cefazolin	30 mcg	12	4	1
5	Cefoxitin	30 mcg	12	–	5
6	Cefotaxime	30 mcg	12	5	–
7	Ceftriaxone	30 mcg	7	9	1
8	Chloramphenicol	30 mcg	14	3	–
9	Ciprofloxacin	5 mcg	9	4	4
10	Co-Trimoxazole	25 mcg	16	1	–
11	Doxycycline hydrochloride	30 mcg	7	6	4
12	Erythromycin	15 mcg	7	6	4
13	Gatifloxacin	5 mcg	13	3	1
14	Gentamicin	10 mcg	13	2	2
15	Kanamycin	10 mcg	11	2	4
16	Penicillin - G	10 units	2	–	15
17	Tetracycline	30 mcg	4	5	8

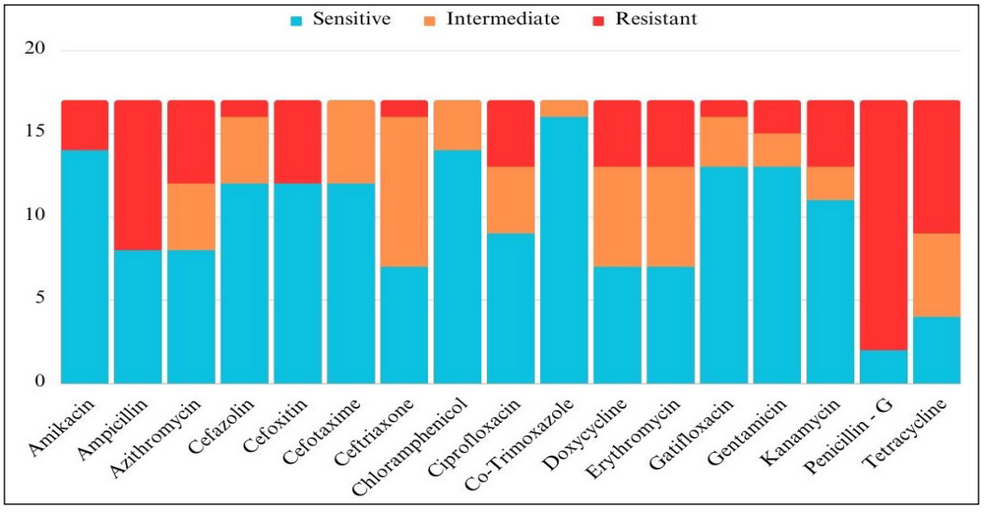

Fig. 3: Anti-microbial susceptibility pattern shown by the isolates

They also reported a prevalence of 80-90% multidrug resistant isolates. Abo-Shama, (2014) investigated *S. aureus* antimicrobial resistance and reported that the isolates showed most resistance to penicillin-G followed by ampicillin, amoxicillin/clavulanic acid, erythromycin, and chloramphenicol.

CONCLUSION

The prevalence of *S. aureus* was found to be 18.8% (n=17/90) in the non-pathological samples obtained from healthy sheep and their environmental samples. The isolates were confirmed by both phenotypic and genotypic

characteristics. Among the isolates, 14 were found to be multi-drug resistant, in that they showed resistance to 3 or more drugs. This indicates the presence of AMR pathogens even in healthy animals and might be due to indiscriminate use of antimicrobials in food production animals. It can be transmitted to other animals in the herd, which might affect the efficacy of treatment protocols, affecting production. It can also get transmitted to human beings via food chain or direct contact, which might impose a public health threat. Hence antimicrobial resistance among such important and common pathogens should be investigated more. This would help in understanding the epidemiology of resistance and in devising quality control measures for judicial use of antimicrobials in animal husbandry practices.

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